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The role of REST and HDAC2 in epigenetic dysregulation of Nav1.5 and nNav1.5 expression in breast cancer

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Abstract

Background: Increased expression of voltage-gated sodium channels (VGSCs) have been implicated with strong metastatic potential of human breast cancer in vitro and in vivo where the main culprits are cardiac isoform Nav1.5 and its 'neonatal' splice variant, nNav1.5. Several factors have been associated with Nav1.5 and nNav1.5 gain of expression in breast cancer mainly hormones, and growth factors.

Aim: This study aimed to investigate the role of epigenetics via transcription repressor, repressor element silencing transcription factor (REST) and histone deacetylases (HDACs) in enhancing Nav1.5 and nNav1.5 expression in human breast cancer by assessing the effect of HDAC inhibitor, trichostatin A (TSA).

Methods: The less aggressive human breast cancer cell line, MCF-7 cells which lack Nav1.5 and nNav1.5 expression was treated with TSA at a concentration range 10–10,000 ng/ml for 24 h whilst the aggressive MDA-MB-231 cells was used as control. The effect of TSA on Nav1.5, nNav1.5, REST, HDAC1, HDAC2, HDAC3, MMP2 and N-cadherin gene expression level was analysed by real-time PCR. Cell growth (MTT assay) and metastatic behaviors (lateral motility and migration assays) were also measured.

Results: mRNA expression level of Nav1.5 and nNav1.5 were initially very low in MCF-7 compared to MDA-MB-231 cells. Inversely, mRNA expression level of REST, HDAC1, HDAC2, and HDAC3 were all greater in MCF-7 compared to MDA-MB-231 cells. Treatment with TSA significantly increased the mRNA expression level of Nav1.5 and nNav1.5 in MCF-7 cells. On the contrary, TSA significantly reduced the mRNA expression level of REST and HDAC2 in this cell line. Remarkably, despite cell growth inhibition by TSA, motility and migration of MCF-7 cells were enhanced after TSA treatment, confirmed with the up-regulation of metastatic markers, MMP2 and N-cadherin.

Conclusions: This study identified epigenetics as another factor that regulate the expression level of Nav1.5 and nNav1.5 in breast cancer where REST and HDAC2 play important role as epigenetic regulators that when lacking enhances the expression of Nav1.5 and nNav1.5 thus promotes motility and migration of breast cancer. Elucidation of the regulatory mechanisms for gain of Nav1.5 and nNav1.5 expression may be helpful for seeking effective strategies for the management of metastatic diseases.

Keywords: Nav1.5, nNav1.5, REST, HDAC2, TSA, Breast cancer, Aggressiveness, MMP2, N-cadherin

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Background

The 'classic' role of voltage-gated sodium channels (VGSC) is to mediate cell regenerative membrane depolarization and conduction of electrical signaling (action potentials) in traditionally 'excitable' cells such as nerve, skeletal, and heart muscle cells [1]. Recent evidences show that VGSCs are also functionally expressed in many types of carcinomas (cancers of epithelial origin), including those of prostate [2, 3], breast [4, 5], lung (small-cell, non-small-cell and mesothelioma) [6–8], cervix [9], ovary [10], and colon [11].

In breast cancer, increased VGSC expression and activity, predominantly the cardiac isoform Nav1.5 and its 'neonatal' splice variant, nNav1.5, correlates positively with metastatic potential in vitro [5]. In vivo, the mRNA expression of Nav1.5 and nNav1.5 was detected in biopsy samples of breast cancer with occurrence of lymph node metastasis [5] and in breast tumors [12]. Ex vivo, using whole-cell patch clamp, cells in breast tumor tissue slices displayed fast inward Na⁺ currents similar to currents detected in the aggressive human breast cancer cell line, MDA-MB-231 [13].

Apparently, a full elucidation of the regulatory mechanisms for such gain of expression of Nav1.5 and nNav1.5 may be helpful for seeking effective strategies for the management of metastatic diseases. At present, various studies have demonstrated that growth factors (epidermal growth factor, nerve growth factor) [14, 15], hormones [16], Na⁺ concentration [17], and VGSC auxiliary β -subunits [18] can all regulate the expression of VGSC in cancer cells thus potentiating aggressiveness. Recently, epigenetic changes has been suggested to contribute to the overexpression of VGSC in aggressive cancer cells [19, 20], though the notion remains to be tested. Herein, the role of epigenetics via transcription repressor, repressor element silencing transcription factor (REST) and histone deacetylases (HDACs) in enhancing Nav1.5 and nNav1.5 expression in human breast cancer cells was assessed.

In classic non-excitable cells i.e. epithelial cell, the expression of a number of genes commonly expressed in neurons including VGSC e.g. Nav1.2 is repressed by the master of transcription repressor, REST [21–23]. REST is a zinc finger protein that binds to a conserved repressor element-1 in a large number of genes encoding fundamental neuronal traits [23] such as ion channels, synaptic vesicle proteins, and neurotransmitter receptors. REST mediates active repression via recruitment of HDACs [24, 25]. With the emergence of neuronal-like adaptation of cancer to gain aggressiveness [26], REST has now proven to play important tumor-suppressor function [27].

HDAC is a chromatin modifying enzyme involved in the removal of an acetyl group on lysine residues of

histone protein contributing to transcriptional silencing of genes [28]. So far, eighteen HDAC isoforms, grouped into four classes have been described in humans [29]. Owing to the discovery of epigenetic drugs such as HDAC inhibitors, cellular functions of HDAC on gene expression is progressively deciphered. Of particular, these drugs have led to better understanding for the role of HDAC in cancers. The best characterised and probably biologically most relevant HDAC in human cancers are class I isoforms HDAC1, HDAC2, and HDAC3 [30]. The expression patterns of HDAC1, HDAC2, and HDAC3 have been evaluated in different types of cancers, including gastric cancer [31], liver cancer [32], prostate cancer [33], breast cancer [34], renal cell cancer [35], ovarian [36] and endometrial carcinomas [37].

This study focused on examining the role of REST, HDAC1, HDAC2, and HDAC3 on influencing the expression of Nav1.5 and nNav1.5 in breast cancer that promote aggressiveness. In doing so, the expression level of Nav1.5, nNav1.5, REST, HDAC1, HDAC2, and HDAC3 were measured and compared between the less aggressive human breast cancer cells, MCF-7 and the aggressive, MDA-MB-231 cells. The effect of HDAC inhibitor, trichostatin A (TSA), on Nav1.5, nNav1.5, HDAC1, HDAC2, HDAC3, REST, MMP2 and N-cadherin gene expression and on cell motility and migration of the less metastatic human breast cancer cells, MCF-7, were investigated.

Methods

Cell culture

MCF-7 and MDA-MB-231 cell line were purchased from the American Type Culture Collection (ATCC) and were cultured in 10 cm or 35 mm plastic culture dishes (Becton–Dickinson, USA). Both of the cells were maintained in Dulbecco's minimum essential medium (DMEM) (Nacalai Tesque, Japan) supplemented with 4 mM L-glutamine (Gibco, USA), 5% of fetal bovine serum (FBS) (Gibco, USA) and 1% penicillin–streptomycin (Gibco, USA). Cultured cells were kept in a humidified incubator at 37 °C, 100% relative humidity and with 5% regulated CO₂.

Chemicals

TSA was purchased from Invivogen, USA and stock solutions were prepared in 100% ethanol (Nacalai Tesque, Japan), sterile filtered and stored at –20 °C until use.

Cell viability and proliferation assays

Briefly, 3 × 10⁴ cells/well were cultured in a 96-well culture plate and incubated at 37 °C for 24 h. The cells were treated with different concentrations of TSA for 24, 48 and 72 h in triplicates. Medium was removed and 100 μ l

of DMEM and 10 μ l of 12 mM 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (Invitrogen, USA) were added to each well and incubated for 4 h. 85 μ l of MTT solution was removed followed by addition of 50 μ l dimethyl sulfoxide (DMSO) (Fisher Scientific, USA). The plate was incubated at 37 °C for 10 min and the absorbance was measured at 540 nm using SpectraMax M5 microplate reader (Molecular Device, USA).

Cell treatment regimes

5×10^5 cells were cultured in 35 mm culture dish and were incubated at 37 °C for 24 h. Media was removed and TSA was added to the cells following the treatment concentration. The cells were incubated for 24 h and preceded for RNA extraction.

Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) analysis

Total RNA was extracted using Sepasol method according to the protocol provided by the manufacturer (Nacalai, Tesque, Japan). Total RNA (1 μ g) was converted to cDNA using the QuantiTect Reverse Transcription Kit (Qiagen, Germany) according to the manufacturer's protocol. Real-time PCR was performed using SensiFAST SYBR Hi-ROX kit (Bioline, UK) according to manufacturer's protocol, in triplicates. Sequence primers used were as follows: β -actin forward, ATGCGGACAGGATGCAGAAG-3' and reverse, 5'-AGAAGCATTTGCGGTGGACG-3'. Nav1.5 forward, 5'-TTGCTTGTTATGGTCATTGGC-3' and reverse, 5'-GTTGTTCATCTCTGTCCAT-3', nNav1.5 forward, 5'-CTGCACGCGTTCACTTTCCT-3' and reverse, 5'-GACAAATTGCCTAGTTTTATATTT-3', HDAC1 forward, 5'-GGAAATCTATCGCCCTCACA-3' and reverse, 5'-TTGCCACAGAACCACCAGTA-3', HDAC2 forward, 5'-CCGTCTACCATGATGATCCTG-3' and reverse 5'-GTCTTCACTGTTCCATCTCCTC-3', HDAC3 forward, 5'-CGCTTCCACTCCGAGGACTA-3' and reverse, 5'-CTGGGCAGTCATCGCCTAC-3', REST forward, 5'-ACTAGACATATGCGTACTCATTTCAG-3' and reverse 5'-CCATTGTGAACCTGTCTTGC-3', MMP2 forward, 5'-TCACTCCTGAGATCTGCAAACAG-3' and reverse 5'-TCACAGTCCGCCAAATGAAC-3', and N-cadherin forward, 5'-GTCAACATCACTCTCACCGA-3' and reverse 5'-ATCCACAGCTCTTATTCTTCCA-3'. Quantitative real-time was performed in an ABI Prism 7000 Sequence Detection System (Life Technologies, USA) and the amplification conditions were as follows: initial activation for 10 min at 95 °C for one cycle, 10 s at 95 °C and 30 s at 60 °C for 34 cycles. Ct values of target genes were normalised to β -actin and the relative mRNA expression of target genes were calculated by the $2^{-\Delta\Delta C_t}$ method [38].

Wound healing assay

Lateral motility of cells was assessed using a monolayer wound healing assay. Cells were plated in 35 mm plastic culture dish at an initial density of 5×10^5 cells/dish and allowed to settle for 24 h prior to wound creation and any pharmacological treatment (T_0). Wounds were created by scratching the monolayer cells using a p200 pipette tip at 90° angle. Wound widths were measured again after 24 h (T_{24}). Lateral motility was calculated according to the motility index formula $1 - (T_{24}/T_0)$ [39] and the percentage of lateral motility was normalised to control (untreated).

Migration assay

Migration assay was performed using transwell chambers with 8 μ m pore inserts (Corning, USA). Briefly, 5×10^4 cells in 300 μ l of 1% serum medium were plated into the upper chambers and 750 μ l medium with 10% serum was added as chemo attractant in lower chambers. After 24 h incubation, non-migrating cells were gently removed using cotton swab and the migrated cells (at the outer surface of membrane insert) were fixed with ice-cold methanol for 15 min. The cells were stained in 0.05% crystal violet for 15 min. Migrated cells were viewed under inverted microscope (10 \times magnification) and images were captured using a monochrome ProgRes CF^{cool} CCD camera (Jenoptik, Germany). Migrated cells in each insert were counted and averaged from 30 random fields.

Statistical analysis

All data are expressed as the mean \pm SEM. Student's *t* test was carried out to evaluate differences between two groups (treated vs untreated). Differences were considered to be significant for values of $p < 0.05$.

Results

MCF-7 cells expressed low level of Nav1.5 and nNav1.5 but higher REST expression

We compared the gene expression level of Nav1.5, nNav1.5 and REST by qRT-PCR in two human breast cancer cell lines, MDA-MB-231 (the highly aggressive human breast cancer cells) and MCF-7 (the less aggressive human breast cancer cells). The expression level of Nav1.5 and nNav1.5 was very low in MCF-7 cells compared to MDA-MB-231 cells. MDA-MB-231 cells expressed 187 ± 31.5 -fold ($p < 0.01$) and 61 ± 20.4 -fold ($p < 0.05$) greater Nav1.5 and nNav1.5 mRNA expression, respectively, compared to MCF-7 cells (Fig. 1a, b). The expression level of REST was significantly lower in MDA-MB-231 cells (0.4 ± 0.03 -fold, $p < 0.00001$) compared to MCF-7 cells.

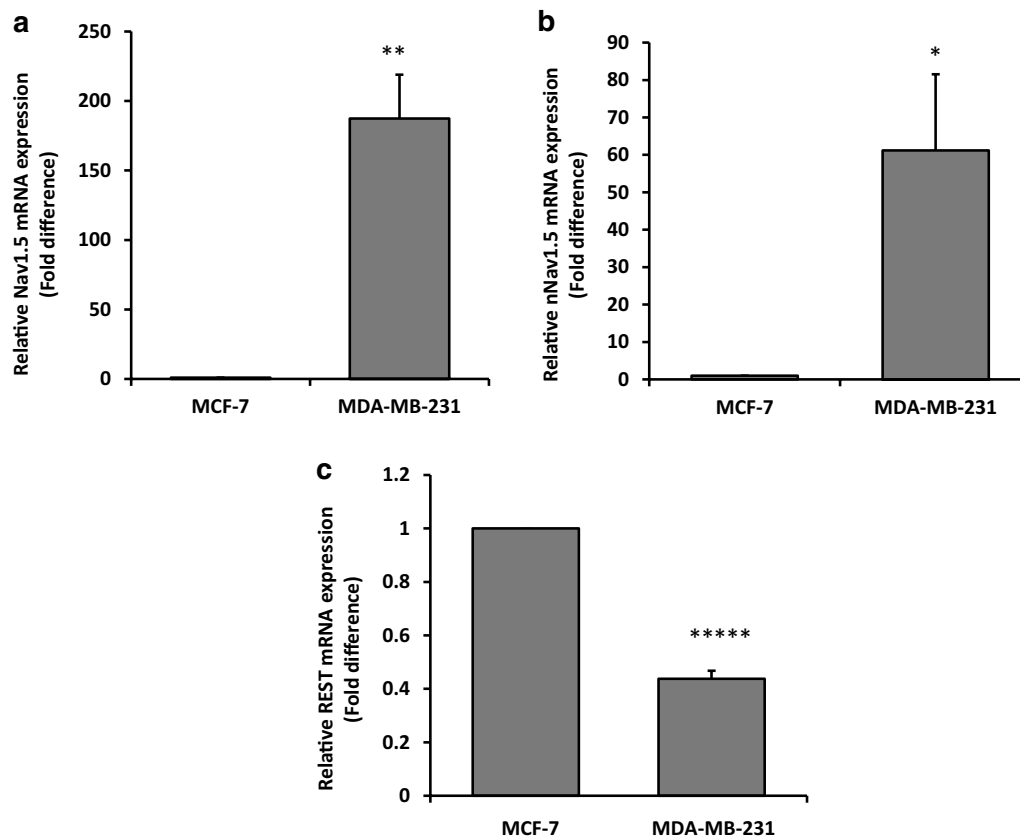


Fig. 1 MCF-7 express low expression of Nav1.5 and nNav1.5 but higher REST expression compared to MDA-MB-231. Relative mRNA expression level of Nav1.5 and nNav1.5 was measured using qRT-PCR where β -actin was used as housekeeping gene. **a** The expression of Nav1.5 in MDA-MB-231 normalised to MCF-7 cells. **b** The expression of nNav1.5 in MDA-MB-231 normalised to MCF-7 cells. **c** The expression of REST in MDA-MB-231 normalised to MCF-7 cells. Data were collected from $n = 3$ independent experiments, presented as mean \pm SEM. Unpaired Student's t test * $p < 0.05$, ** $p < 0.01$, and **** $p < 0.00001$

MDA-MB-231 cells expressed low level of HDAC1, HDAC2, and HDAC3

We measured the basal expression levels of HDAC1, HDAC2 and HDAC3 in MDA-MB-231 cells compared to MCF-7 cells (without TSA treatment). As presented in Fig. 2, HDAC1, HDAC2 and HDAC3 exhibited lower mRNA expression in MDA-MB-231 compared to MCF-7 cells. However, only HDAC2 showed a significantly lower expression in MDA-MB-231 cells ($p < 0.05$).

TSA increased the mRNA expression level of Nav1.5 and nNav1.5 in MCF-7 cells

Next, we examined the effect of TSA treatment on Nav1.5 and nNav1.5 mRNA expression by qRT-PCR. In comparison to untreated cells, our results showed that treatment with 1000 and 10,000 ng/ml TSA for 24 h significantly increased the expression of Nav1.5 by 26 ± 7.0 -fold ($p < 0.05$) and 39 ± 5.1 -fold ($p < 0.01$), respectively (Fig. 3a and b). Similarly, the expression of nNav1.5 was increased

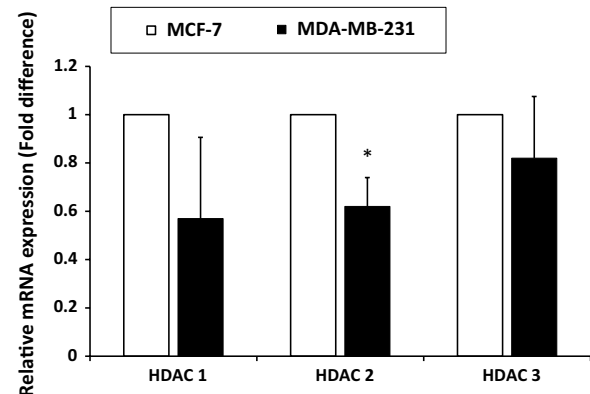
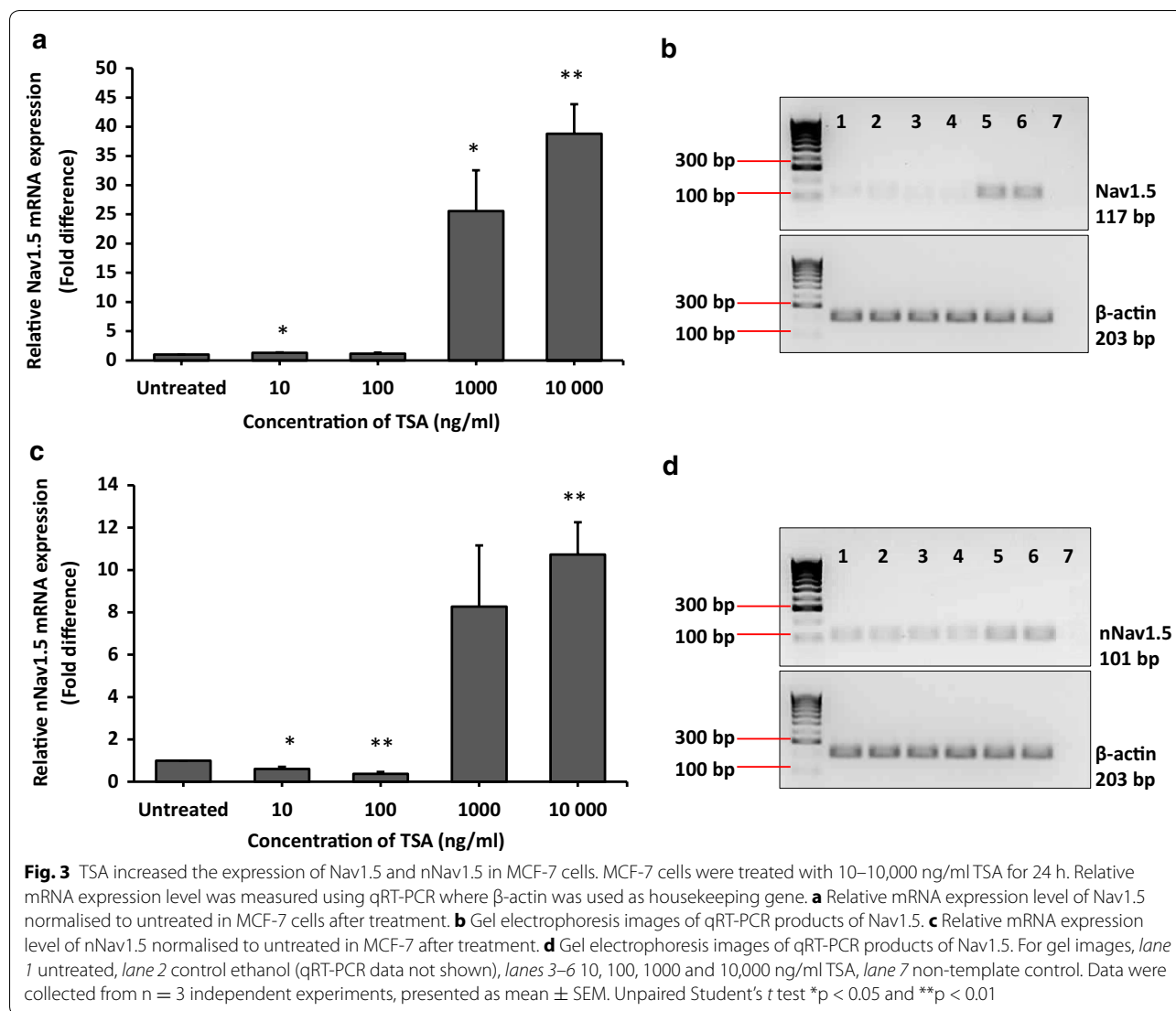


Fig. 2 HDAC2 is significantly lower in MDA-MB-231 cells compared to MCF-7 cells. Relative mRNA expression level of HDAC1, HDAC2 and HDAC3 was measured using qRT-PCR where β -actin was used as housekeeping gene. mRNA expression of each HDAC in MDA-MB-231 was normalised to HDAC in MCF-7 cells. Data were collected from $n = 3$ independent experiments, presented as mean \pm SEM. Unpaired Student's t test * $p < 0.05$



by 8 ± 2.9 -fold and 11 ± 1.5 -fold ($p < 0.01$) with 1000 and 10,000 ng/ml TSA, respectively (Fig. 3c and d).

TSA reduced the mRNA expression level of REST in MCF-7 cells

In comparison to untreated cells, our results showed that gene expression of REST was significantly down-regulated to 0.8 ± 0.1 ($p < 0.05$), 0.3 ± 0.1 ($p < 0.001$) and 0.2 ± 0.03 ($p < 0.0001$) with increasing dose of 100, 1000 and 10,000 ng/ml TSA, respectively (Fig. 4).

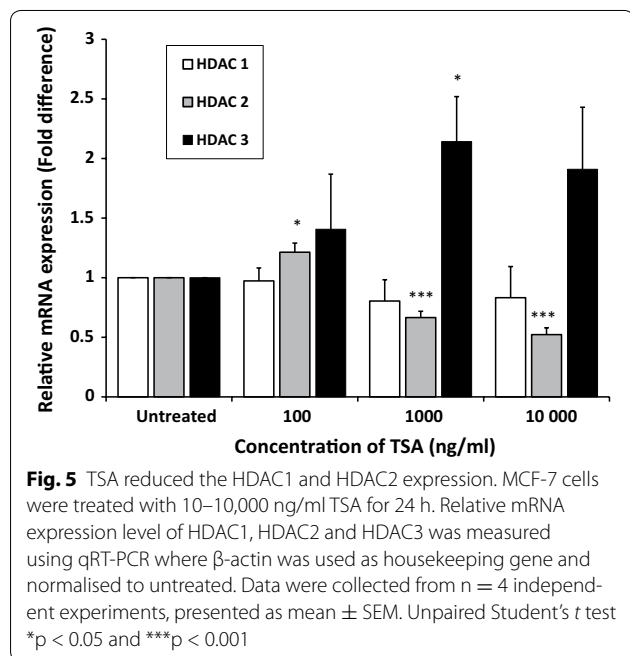
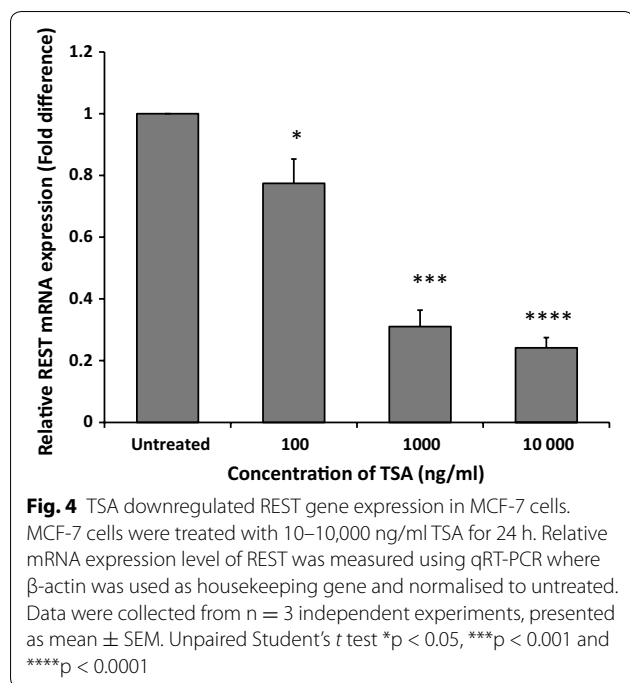
HDAC2 mRNA expression level was downregulated by TSA in MCF-7 cells

Next, the effect of TSA on the mRNA expression of HDAC1, HDAC2 and HDAC3 in MCF-7 cells were analysed by qRT-PCR. The expression of HDAC1 was reduced but not significant, with concentrations of

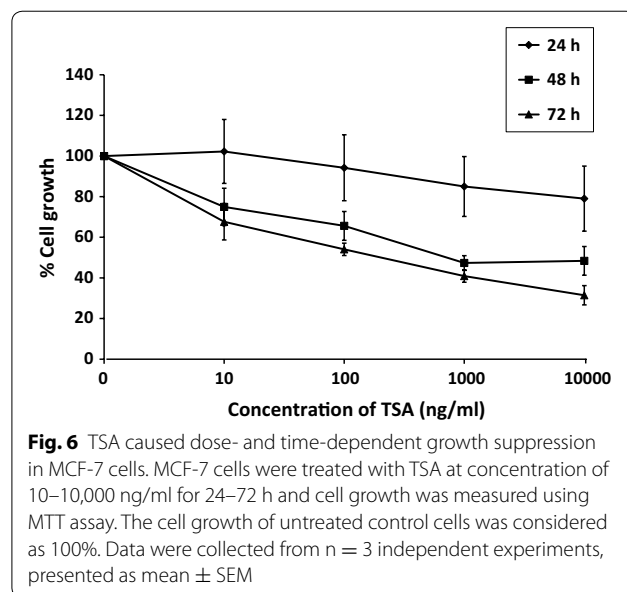
100–10,000 ng/ml TSA by 0.97 ± 0.1 -fold, 0.8 ± 0.2 -fold and 0.83 ± 0.3 -fold, respectively. HDAC2 expression was significantly down-regulated by $\sim 0.6 \pm 0.1$ -fold ($p < 0.001$) and $\sim 0.5 \pm 0.1$ -fold ($p < 0.001$) with concentrations of 1000 and 10,000 ng/ml TSA, respectively. In contrast, the expression of HDAC3 was significantly increased by 2 ± 0.4 -fold ($p < 0.05$) with 1000 ng/ml TSA (Fig. 5).

TSA caused growth suppression in MCF-7 cells

To determine the effect of TSA on cell growth, MCF-7 cells were treated with 10–10,000 ng/ml of TSA for 24, 48 and 72 h. As presented in Fig. 6, cell growth was reduced in dose- and time-dependent manner by TSA. Cell growth at 24, 48, and 72 h was reduced by 21 ± 16.0 , 52 ± 7 and $69 \pm 4.7\%$, respectively, with 10,000 ng/ml TSA.



TSA enhanced lateral motility and migration of MCF-7 cells
 To investigate the effect of TSA on cell metastatic behaviors of MCF-7 cells, we performed wound healing and migration assay. As shown in Fig. 7, cell motility was enhanced by $\sim 20 \pm 8.2\%$ and $\sim 90 \pm 40.2\%$ at concentration of 100 and 1000 ng/ml TSA, respectively. However, at 10,000 ng/ml TSA, motility was only enhanced



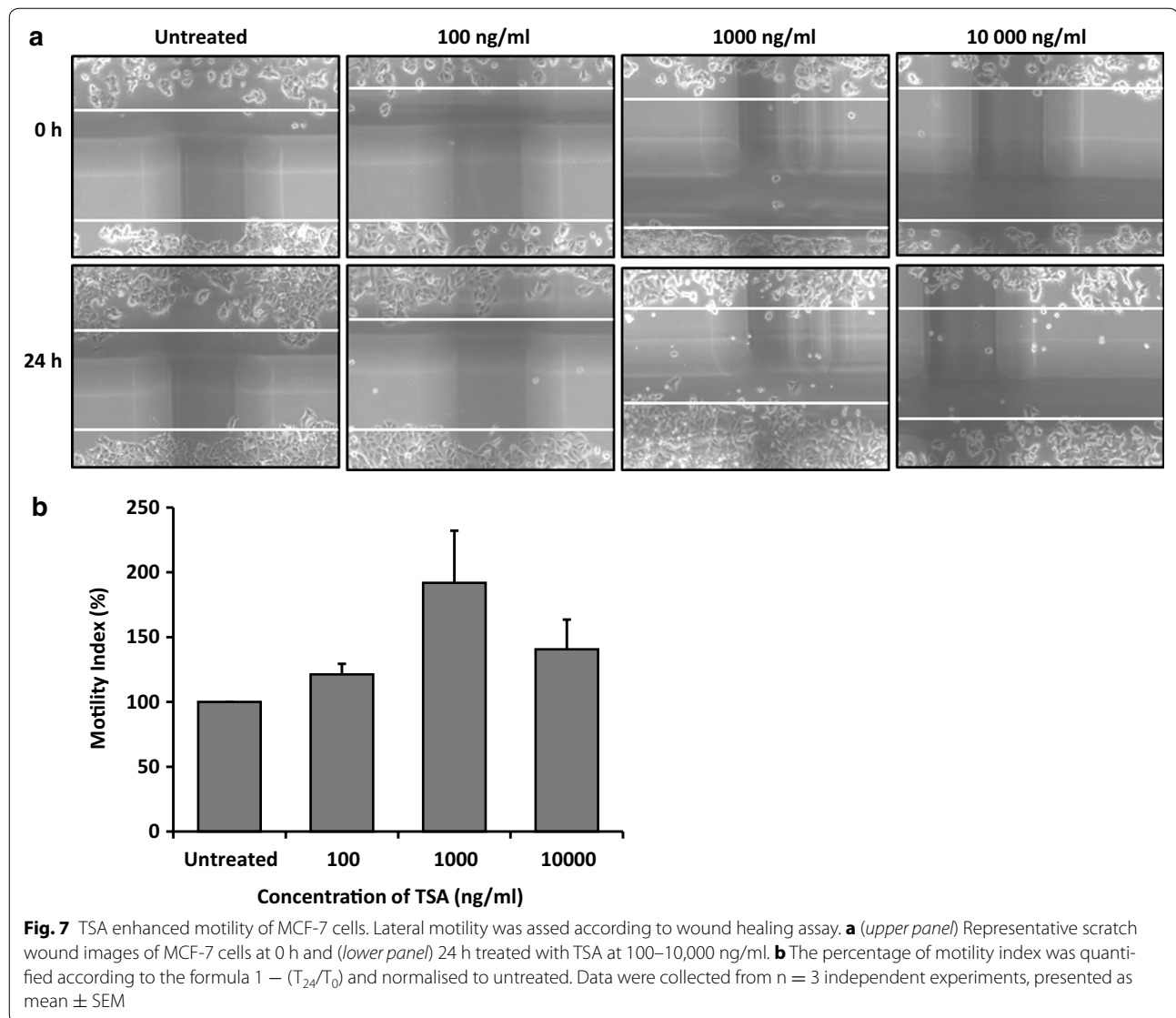
by $40 \pm 23.0\%$, compared to the untreated cells due to toxicity effect. Similarly, our data revealed that TSA at 1000 ng/ml significantly enhanced the migration of MCF-7 cells by $\sim 300 \pm 117.5\%$ ($p < 0.05$) (Fig. 8). Again, at 10,000 ng/ml TSA, migration was only enhanced by $107 \pm 41.1\%$ ($p < 0.05$), compared to the untreated cells due to growth inhibition effect.

mRNA expression of MMP-2 and N-cadherin was upregulated by TSA

The mRNA expression of two metastasis markers, MMP2 and N-cadherin was investigated to confirm increased in metastatic behaviours in MCF-7 cells by TSA. We found that mRNA expression of MMP2 was significantly upregulated by $\sim 16 \pm 2.8$ -fold ($p < 0.01$) and 22 ± 2.9 -fold ($p < 0.01$) with concentrations of 1000 and 10,000 ng/ml TSA, respectively (Fig. 9a and b). Similarly, N-cadherin mRNA expression was also enhanced by 2.8 ± 0.6 -fold ($p < 0.05$) and 5.3 ± 1.4 -fold ($p < 0.05$) at the same concentrations of TSA where MMP2 mRNA expression increased (Fig. 9c and d).

Discussion

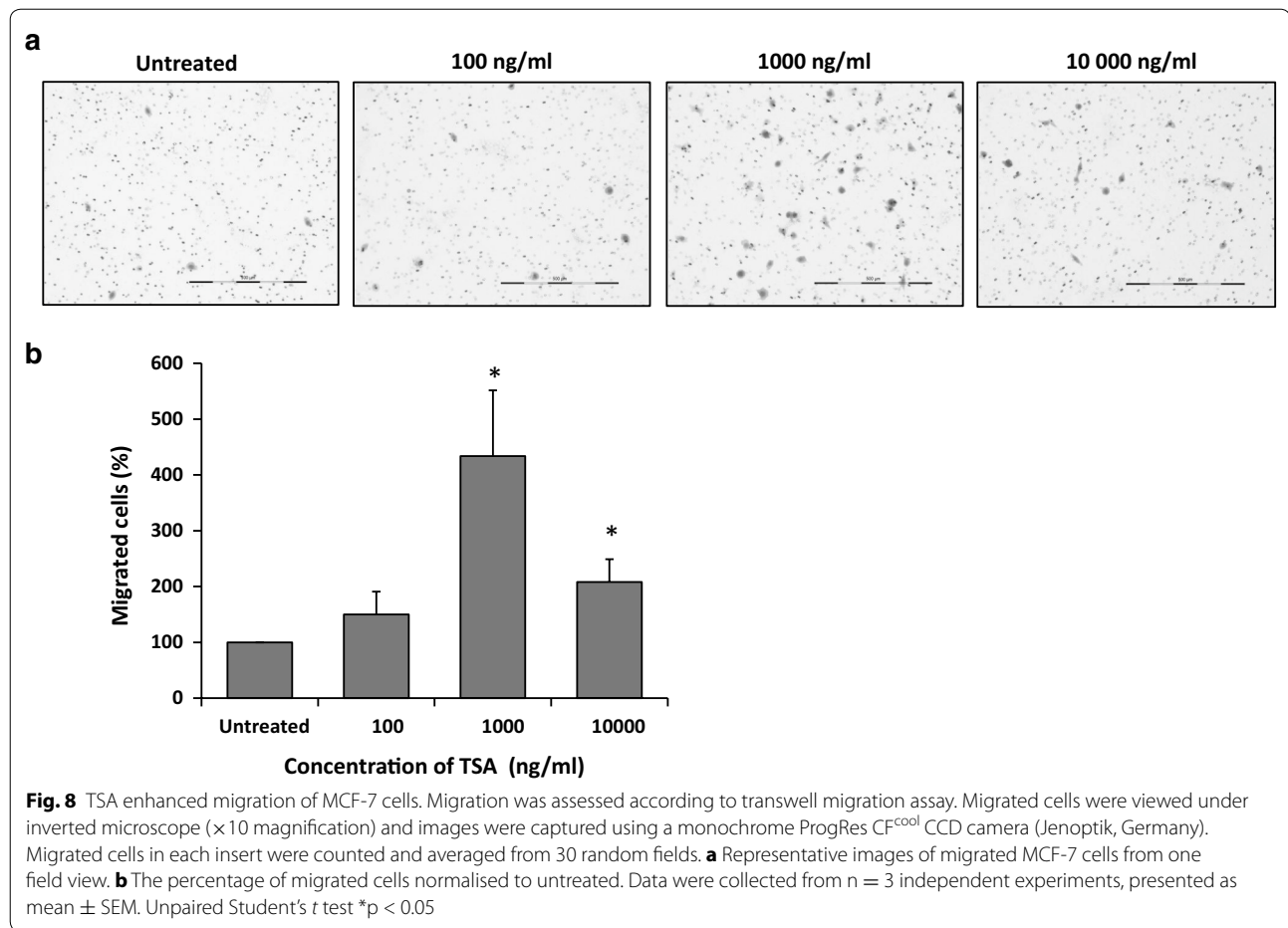
In search to understand factors that contribute to the enhancement of VGSC expression in aggressive cancers, reports have demonstrated that the primary regulators are hormones [16], growth factors [14, 15], auxiliary β -subunits [18] and auto-regulation via Na^+ concentration [17, 38]. Though studies have shown that VGSCs are highly expressed in various tumour types with no alteration of their genetic code [19, 20], epigenetic regulation of VGSCs in these cancers is not known.



To our knowledge, this is the first investigation on the regulatory role of epigenetic regulators, REST and HDACs on the expression of Nav1.5 and nNav1.5 in breast cancer that promote aggressiveness.

Firstly, we showed that less aggressive human breast cancer cells, MCF-7 expressed significantly lower level of Nav1.5 ($p < 0.01$) and nNav1.5 ($p < 0.05$) mRNA expression than aggressive human breast cancer cells, MDA-MB-231. Inversely, basal mRNA expression level of REST, HDAC1, HDAC2 and HDAC3 was higher in MCF-7 compared to MDA-MB-231 cells. In excitable cells e.g. neurons, REST dependence of VGSC expression suppression has been reported and confirmed several time [22, 40–42]. However in cancers, increased expression of Nav1.5/nNav1.5 and lack of REST expression

level have been separately reported to be associated with breast cancer aggressive phenotype [5, 39, 40, 43]. Meanwhile, in adult heart, inhibition of REST resulted in re-expression of various neonatal genes including those encoding 'neonatal' ion channels such as the hyperpolarization-activated, cyclic nucleotide-gated channels and T-type Ca^{2+} channels [44]. From here we postulated REST-Nav1.5/nNav1.5 interrelation in breast cancer. With extensive reports demonstrating REST epigenetic regulation is dependent on its ability to recruit HDACs for transcriptional repression activity [45], therefore, higher mRNA expression level of HDAC1, HDAC2, and HDAC3 in MCF-7 were expected. Recent study showed that HDAC1 was highly expressed in hormone receptor positive breast tumor [34] which linked to MCF-7 cells,



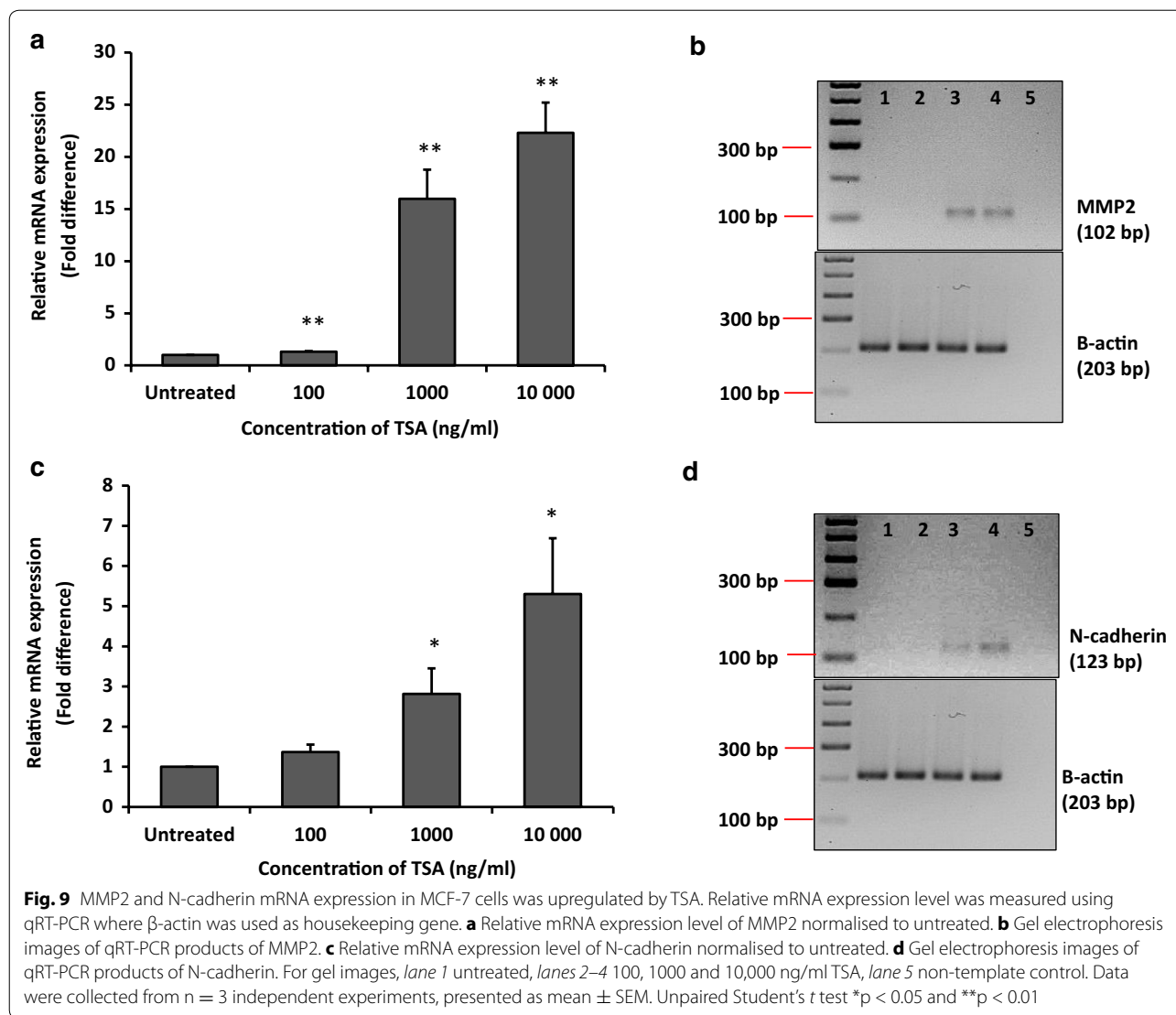
a human breast cancer cell line widely used for in vitro model with positive estrogen receptor. Our postulation extended to REST-HDAC-Nav1.5/nNav1.5 interrelation in breast cancer.

Studies have shown HDAC inhibitors are able to restore the expression of VGSC isoforms in certain pathological conditions arisen from abnormally repression of VGSCs. For example, TSA was demonstrated to re-express Nav1.8 protein expression in dorsal root ganglion of nerve injury-induced neuropathic pain [46]. Similarly, with another famously known HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA), re-expression of Nav1.5 protein expression was achieved in Duchenne muscular dystrophic mice model which lack Nav1.5 [47]. Additionally, with another known HDAC inhibitor, valproic acids (VPA), chronic treatment with VPA up-regulates the mRNA and cell surface expression of Nav1.7 in adrenal chromaffin cells [48]. Herein, TSA treatment resulted in a significant increase of Nav1.5 and nNav1.5 mRNA expression level in MCF-7 cells ($p < 0.01$ at 10 000 ng/ml TSA). Subsequently, the effect of TSA on HDACs and REST gene expression were measured to ensure that

the observed changes in Nav1.5 and nNav1.5 expression stemmed from REST/HDACs targeting. Indeed, mRNA expression level of REST, HDAC1 and HDAC2 in MCF-7 cells was downregulated by TSA (though only effect on REST and HDAC2 was statistically significant, $p < 0.05$).

In normal epithelial cells, REST is expressed abundantly and functions as tumor suppressor [49] where lack of REST have been implicated in carcinomas of breast [50], colorectal [27], and small cell lung [51]. In fact, REST is shown to be lost in breast tissues of patients with aggressive phenotype (significant poor prognosis and more than twice as likely to undergo disease recurrence within the first 3 years after diagnosis) [52]. The significantly lower REST expression detected in aggressive Nav1.5/nNav1.5-expressing MDA-MB-231 cells compared to less aggressive MCF-7 and REST downregulation followed by up-regulation of Nav1.5/nNav1.5 after TSA treatment in MCF-7 cells support our hypothesis of REST interrelation with Nav1.5/nNav1.5.

Meanwhile, reports demonstrated that HDAC1 and HDAC2 have a high degree of homology and are able to form a complex in the nucleus [45, 46] which explain



the similar effects obtained by TSA on both molecules in MCF-7 cells. In our model of less aggressive breast cancer, the significant reduction of HDAC2 expression was in accordance to colon cancer where HDAC2 was reported to share similar feature to those of tumor-suppressor genes when mutation-loss of HDAC2 function lead to oncogenesis [53].

On the contrary, HDAC3 expression was significantly increased after TSA treatment ($p < 0.05$). The increased pattern of HDAC3 gene expression level by TSA in MCF-7 cells was similar to that reported by Duong et al. [54] using the similar cell line. Opposite to HDAC1/HDAC2, overexpression of HDAC3 in breast cancer is associated with clinicopathological indicator of disease progression [34], which is in line with our result, HDAC3 increased of expression after treatment with TSA in

MCF-7. The role of HDAC3 as tumour suppressor gene is only recognisable in hepatocellular carcinomas, where previously, Bhaskara et al. [55] observed that liver-specific HDAC3 knockdown resulted in overt hepatocellular carcinomas. Thus, our results support the role of HDAC2 as another epigenetic regulator for Nav1.5 and nNav1.5 expression in breast cancer.

TSA was first recognized for its anticancer activity via cell cycle arrest and apoptosis in several types of cancer including breast cancer [51, 52]. The drug has been reported to induce caspase activity and apoptosis in MCF-7 cells via a cytochrome *c*-dependent pathway [56]. Unfortunately, in clinical experiments, HDAC inhibitors including TSA seem to have serious limitations which hindered their therapeutic potential. For example, the current two structurally distinct HDAC

inhibitors – SAHA (vorinostat, Zolinza™) and FK228 (romidepsin, Istodax™), both are reported to have not been effective in clinical trials involving solid tumors i.e. refractory breast, colorectal, non-small cell lung and thyroid cancers [57] and most importantly, these drugs cause serious cardiac toxicity [58].

In this study, despite of growth inhibition by TSA, remarkably, motility and migration ($p < 0.05$) of MCF-7 cells were still significantly increased. These findings were in line with several other studies which demonstrated similar enhancement of metastatic parameters e.g. invasion and migration by TSA in various human cancers in vitro including those of neuroblastoma, meningioma, and prostate [59], rhabdomyosarcoma [60], breast, gastric, liver, and lung cancer cell lines [61]. In fact, in vivo, treatment with TSA also significantly promotes metastasis in nude mice [61]. Subsequently, the underlying mechanism of metastatic enhancement by TSA was reported to be due to up-regulation of metastatic related markers e.g. urokinase plasminogen activator [59], PKCs [61] and Ezrin [60]. Additionally, TSA also led to prostate cancer aggressiveness via induction of epithelial-to-mesenchymal transition phenotype which associated with increased expression of transcription factors ZEB1, ZEB2 and Slug, and mesenchymal markers such as vimentin, N-cadherin and fibronectin [62]. As in the present study, enhancement of motility and migration of MCF-7 cells by TSA were likely due to the increased expression level of Nav1.5 and nNav1.5. Both Nav1.5 and nNav1.5 are already recognised as a potent metastatic gene in potentiating breast cancer metastatic parameters i.e. motility, migration and invasion when specific VGSC blocker, TTX and other VGSC blockers e.g. phenytoin, ranolazine have all precisely suppressed breast cancer metastasis in vitro and in vivo even at dose concentration that does not interfere with proliferation [5, 13, 63–66]. Enhanced motility and migration of MCF-7 cells after TSA treatment were also pivotal to support the functional re-expression of Nav1.5/nNav1.5. Importantly, our findings could explicate cellular mechanisms for the previous disappointments in clinical experience with HDAC inhibitors in patients with solid tumors.

Conclusions

Overall, our study demonstrated that epigenetics play role in controlling Nav1.5/nNav1.5 expression breast cancer. Downregulation of REST and HDAC2 expression level by TSA lead to enhanced Nav1.5 and nNav1.5 expression that transformed the less aggressive, MCF-7 cells to gain aggressiveness. We postulated that when REST and HDAC2 are lacking in breast cancer, enhance expression of Nav1.5 and nNav1.5 promotes aggressiveness.

Abbreviations

VGSCs: voltage-gated sodium channels; HDACs: histone deacetylases; TSA: trichostatin A; HDACis: histone deacetylase inhibitors; Nav1.5: voltage-gated sodium channel 1.5; nNav1.5: neonatal splice variant of Nav1.5; HDAC1: histone deacetylase 1; HDAC2: histone deacetylase 2; HDAC3: histone deacetylase 3; MMP2: matrix metalloproteinase 2.

Authors' contributions

NSK and HDD carried out the molecular biology studies, performed the statistical analysis and drafted the manuscript. NFM and NSY conceived the study, and participated in its design and coordination and LCY helped to draft the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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